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ORIGINAL ARTICLE

Life history and host specificity of the Japanese flea beetles *Trachyaphthona sordida* and *T. nigrita* (Coleoptera: Chrysomelidae), potential biological control agents against skunk vine, *Paederia foetida* (Rubiaceae), in the southeastern parts of the United States and Hawaii

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Abstract

Skunk vine, *Paederia foetida* (Rubiaceae), is native to Asia and has been recognized as an invasive weedy vine of natural areas in Florida and Hawaii. Two insects, *Trachyaphthona sordida* and *Trachyaphthona nigrita* (Coleoptera: Chrysomelidae) from Japan are being considered as potential biological control agents against skunk vine. To gather fundamental information on their biology, we carried out field surveys and laboratory experiments in Kyushu, southern Japan, between 2003 and 2006. We found that *T. sordida* is commonly distributed in Kyushu and *T. nigrita* is restricted to the southern parts of Kagoshima Prefecture on the southern part of Kyushu. These species are fundamentally univoltine and adults appear in late April to early July. *Trachyaphthona sordida* overwinters as mature larvae and *T. nigrita* as mature larvae or rarely as adults. Larvae of both species feed on fine roots of *P. foetida* in the field and *Serissa foetida* (Rubiaceae) under rearing conditions, and they appear to have tribe-level host specificity in their host range. On the basis of these results, we suggest that both species are suitable as biological control agents.

Key words: Alticinae, biological control, host specificity, life history, *Paederia foetida*, skunk vine, *Trachyaphthona*.

INTRODUCTION

Skunk vine, *Paederia foetida* Linnaeus (Rubiaceae) (= *Paederia scandens*, *Paederia chinensis*), is a weedy vine that is widely distributed in Japan, Taiwan, central to southern China, Indochina, Burma, the Himalayas, India, and Malaysia (Yamazaki 1993). Throughout our field surveys, we have observed this species growing in sunny meadows, on grassy hillsides, in forest shade, on waste ground, in hedges, in thickets, on roadsides and along fences. The large native range and the diversity of climatic zones and habitats occupied indicate that skunk vine can tolerate a broad range of climatic, hydrological and edaphic conditions (Gann & Gordon 1998).

Skunk vine is a recently recognized weed of natural areas in Florida, USA, and is spreading into other parts of the southern United States (Pemberton & Pratt 2002). The weed was reportedly introduced as a potential fiber plant to an unknown location in Florida by the United States Department of Agriculture (USDA) prior to 1867 (Morton 1976). The weed is distributed from Texas to North Carolina in the USA (Pemberton & Pratt 2002). Skunk vine is a category I Florida Exotic Pest Plant

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Council weed (Langeland & Burks 1998), a listing that groups the plant with the most invasive weed species in Florida. In Hawaii, skunk vine has naturalized and is known from the islands of Hawaii, Oahu and Kauai (Puff 1991).

Skunk vine can create dense canopies leading to damage or death of native vegetation (Gann & Gordon 1998). Direct damage to overstory plants increases the probability of gap formation and may alter the impact of fire, which occurs in many of the invaded communities (Gann & Gordon 1998). On the island of Hawaii, *P. foetida* is a very serious weed in nurseries producing ornamental foliage plants. The weed infests field plantings used for propagation. Control of the weed is very difficult because stock plants are easily injured if herbicides are applied. At times, growers have had to abandon or destroy stock plants that have become overgrown by skunk vine. Stocker and Brazis (1999) estimated the cost of manually removing *P. foetida* from a moderately infested area at US$1622/ha. Estimates for herbicidal treatments of light (5.1 vines per m^2) and moderate (33.6 vines per m^2) infestation levels are US$430/ha and US$645/ha, respectively (B. Nelson, pers. comm., 2000). Herbicide treatments are expensive, and also pose a risk to non-target plants (e.g. Luken *et al.* 1994). Because complete control is not achieved with a single treatment, regardless of the method, additional control efforts including classical biological control must be undertaken.

A classical biological control program was initiated by Florida state agencies and the USDA Agricultural Research Service. The weed was thought to be a good target for biological control because it is taxonomically isolated: both the genus *Paederia* and tribe Paederiae are absent from native floras in both the continental USA and Hawaii, and only one minor ornamental plant *Serissa foetida* (Linnaeus) Lam in the tribe Paederiae is present. In addition, there was also evidence of the existence of natural enemies of *Paederia* (Pemberton & Pratt 2002). After surveying literature, Pemberton in 1997 and Pemberton and Pratt in 2002 made visits to Japan to discover herbivores associated with skunk vine. Subsequently, a cooperative project was developed with the Entomological Laboratory of Kyushu University to study two Japanese flea beetles, *Trachyaphthona sordida* (Baly) and *Trachyaphthona nigrita* Ohno (Coleoptera: Chrysomelidae), which appeared to be promising biological control agents against the weed.

Trachyaphthona species are known from Japan, Taiwan, China and Indochina (Ohno 1961). *Trachyaphthona sordida, T. nisii* (Ohno) and *T. nigrita* (including a subspecies, *T. n. maebarai* Ohno) have been described from Japan. Kimoto (1966) included *T. nigrita* in *Aphthona*, but Ohno (1961, 1966) placed *T. nigrita* in *Trachyaphthona*. We treat it as *T. nigrita* based on the species identification by Mr H. Matsuzawa (pers. comm., 2004). *Trachyaphthona sordida* has been found on Hokkaido, Honshu, Shikoku, Kyushu, and Okinoshima, Iki, Tsushima, Goto and Yakushima Islands, Japan (Kimoto & Takizawa 1994). *Trachyaphthona nigrita* has been found on Kyushu, Yakushima Island, the Tokara Islands, Amamioshima Island, Okinoerabu Island, Okinawa Island and the Kerama Islands, Japan (Kimoto & Takizawa 1994).

The genus *Trachyaphthona* belongs to the subfamily Alticinae and larvae of some species are root feeders (Frick 1970; Gassmann *et al.* 1996; Julien & Griffiths 1998). The feeding site of alticine larvae varies, for example, they can feed on or within leaves, within stems or on or within roots in the soil (Kobayashi 2000). Larvae mature at the third larval stadium and pupate in the soil. Many species overwinter as adults (Kimoto & Takizawa 1994). Due to difficulties in observing larvae in the soil, only limited information is available on their feeding behavior. We have observed adults of the Japanese *Trachyaphthona* beetles feeding on leaves and stems of *P. foetida* and suspect that their larvae might be root feeders, like other Alticinae beetles, but details of their life history traits have never been intensively studied. In the present paper, we report on their life history and host specificity with the aim of evaluating their suitability as biological control agents against skunk vine.

MATERIALS AND METHODS

Life history

Field survey: distribution and seasonal occurrence

Trachyaphthona sordida was surveyed in the northern prefectures (Fukuoka, Saga, Nagasaki and Ôita) and southern prefectures (Kagoshima and Kumamoto) of Kyushu from early May to November during 2003–2006. Most *T. sordida* adults were located by visual searches then collected with aspirators from leaves of *P. foetida* that was growing near the ground under relatively humid conditions without direct sunshine. Some adults were collected by beating young *P. foetida* plants. The collection data for northern and southern Kyushu were summed separately, because annual mean temperature in Fukuoka is approximately 2°C lower than in Kagoshima (Japan Meteorological Agency 2006), where

Entomological Science (2008) **11**, 143–152

T. sordida adults would appear earlier than in northern Kyushu. To compare field data on different census dates with different survey efforts, the total number of *T. sordida* adults collected on each census date was converted to the average number of adults per person per hour. Then, the average number was plotted against every census date.

Trachyaphthona nigrita adults were collected from various places in Kagoshima prefecture (localities 13–21 in Fig. 1) from late April to November during 2004–2006. Most *T. nigrita* adults were collected by beating well-grown *P. foetida* that bore thick and large leaves in sunny places. As with *T. sordida*, the average number of *T. nigrita* adults per person per hour was plotted against each census date.

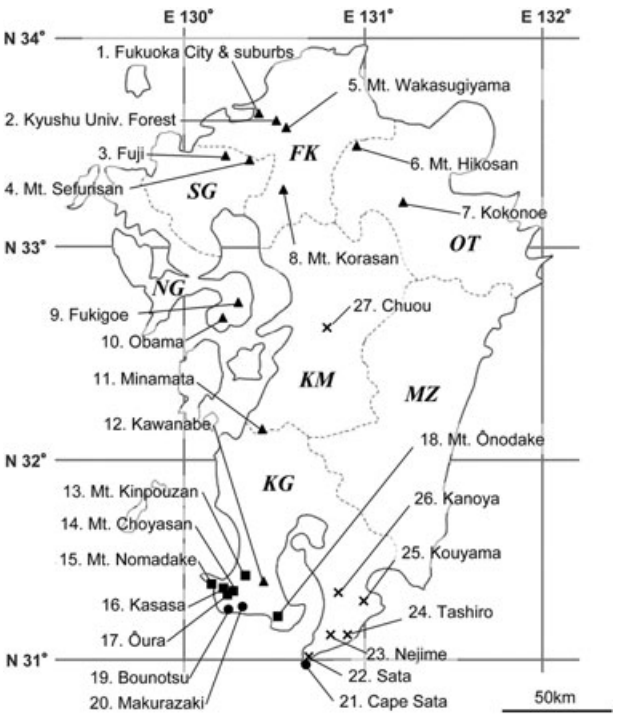

Figure 1 Map of Kyushu showing the localities where adults were collected between 2003 and 2006. Capital italic letters indicate the names of prefectures: *FK*, Fukuoka; *KG*, Kagoshima; *KM*, Kumamoto; *MZ*, Miyazaki; *NG*, Nagasaki; *OT*, Oita; *SG*, Saga. Species collected: (▲), *Trachyaphthona sordida*; (●), *Trachyaphthona nigrita*; (■), both *T. sordida* and *T. nigrita*; (×), neither *T. sordida* nor *T. nigrita*. We used the old names of cities and towns before 2005 to express the localities where we surveyed or referred to collection records, because the localities can be more easily identified using the old names derived from restricted areas than the new names given to unified cities and towns. Each locality in Kyushu is expressed in the text using the name of the city or town together with a numeral that indicates the location of the locality on the map.

Laboratory experiments

Paederia foetida *used for experiments*. From April to May in 2004, 2005 and 2006, *P. foetida* vines (30–50 cm long) were transplanted from the Hakozaki Campus of Kyushu University (locality 1 in Fig. 1) to paper pots (8 cm in length, 8 cm in width and 7.5 cm in height) or plastic pots (10 cm in diameter and 9 cm in height). They were kept out of doors without direct sunshine and watered every day. Insect larvae were fed with fine roots obtained from these plants and roots were renewed every other day. Adults of *Trachyaphthona* beetles were fed with *P. foetida* leaves collected from the same campus.

Life history studies. Prior to the larvae-rearing experiments, females of both *T. sordida* and *T. nigrita* were kept in a plastic box (7.5 cm in length, 7.5 cm in width and 10 cm in height) containing soil to a depth of approximately 1.5 cm in order to confirm egg-laying locations.

Adults of *T. sordida* and *T. nigrita* collected were kept in plastic Petri dishes (9 cm in diameter and 1.5 cm in height) containing a sheet of moistened filter paper (hereafter, "Petri dish" refers to this arrangement) and fed with *P. foetida* leaves. Eggs laid on the moistened paper were transferred to different Petri dishes. Eggs and larvae were incubated under various conditions to study their life history traits, such as the number of larval stadia, the duration of the egg stage and each larval stadium, lower developmental threshold, and day degrees required for the development of eggs and larvae of the various stadia. All eggs and larvae were kept under dark conditions because they live in the soil.

Trachyaphthona sordida. In 2004 and 2005, adults of *T. sordida* collected from localities 1–8 and 11–18 in Figure 1 were kept under ambient laboratory conditions. Eggs laid were kept in an incubator at 22°C in 2004 and at 15, 20 and 25°C in 2005. In 2004, hatched larvae were kept in an incubator at 22°C. In 2005, hatched larvae were transferred to Petri dishes containing soil on a sheet of moistened paper and kept in an incubator at 15, 18, 20 or 25°C.

Trachyaphthona nigrita. Adults of *T. nigrita* collected from localities 13–20 (Fig. 1) were kept under ambient laboratory conditions in 2005 and in an incubator at less than 25°C under conditions of 14 h light : 10 h dark (L : D 14:10) in 2006. Eggs laid were kept in an incubator at 15, 20 and 25°C in 2005 and 22.5°C in 2006.

In 2005, hatched larvae were transferred to Petri dishes containing soil and kept in an incubator at 15, 18, 20 or 25°C. In 2006, hatched larvae were transferred individually to a plastic box (20 mm in length, 20 mm in width, and 15 mm in height) containing soil on a piece of moistened paper (hereafter, "plastic box" refers to this arrangement). Each box containing a larva was covered with Parafilm and kept in an incubator at 22.5°C. As will be mentioned later, this experiment was designed also to assess the amount of *P. foetida* roots eaten by *Trachyaphthona* larvae, which was facilitated by rearing larvae individually in transparent plastic boxes.

Consumption of P. foetida *root by* Trachyaphthona *larvae*. Adults of both *T. sordida* and *T. nigrita* collected from localities 5, 6, 8 and 15–19 (Fig. 1) in June 2006 were kept separately. *Trachyaphthona sordida* and *T. nigrita* were incubated under L : D 14:10 conditions at 22.5°C and 25°C, respectively. Eggs of both species were separately kept in an incubator at 22.5°C. Hatched larvae were transferred individually to a plastic box. Each box containing a larva was covered with Parafilm and kept in an incubator at 22.5°C. The first, second and third instars of *T. sordida* were fed with one, three and five pieces of fine *P. foetida* root (each piece 1 mm in diameter and 15 mm in length), respectively, which were renewed every other day. Similarly, the first, second, third and fourth instars of *T. nigrita* were fed with one, three, five and six pieces of fine *P. foetida* root (each piece the same size as for *T. sordida*), respectively, which were renewed every other day.

The length and diameter of *P. foetida* fine roots left uneaten by the larvae were measured using a micrometer under a binocular microscope every other day when the roots were renewed, and the percentage of *P. foetida* root eaten by each larva was estimated. The data were summed to calculate the total amount of roots eaten by all larvae during particular larval stadia. Then, the mean amount of root eaten by each larva during each larval stadium was calculated.

Host specificity

Plant species used

We used *Serissa foetida* (Linnaeus) Lam (= *S. japonica* (Thunberg) Thunberg cv. Dancyouge) and *Morinda citrifolia* Linnaeus (Rubiaceae) for the experiment. *Serissa foetida* is a small ornamental shrub that is native to Japan. It belongs to the tribe Paederieae, like *P. foetida* (Bremer & Manen 2000). *Morinda citrifolia* is widely distributed in South-East Asia and has cultural value in Hawaii. It belongs to the tribe Morindeae, unlike *P. foetida* (Bremer & Manen 2000). No native species of the tribe Paederieae grow either in Florida or in Hawaii, and *S. foetida* and *M. citrifolia* are the only cultivated members of the tribe used as economic plants. If biological control agents used for skunk vine have genus-level host specificity, no native plants or economic plants will be infested. More extensive testing using plants representing the many other tribes in the Rubiaceae would be needed to more precisely determine the host ranges of these beetles.

In June 2005 and 2006, small plants of *S. foetida* (30–40 cm in height) and *M. citrifolia* (15–20 cm) were obtained from commercial nurseries and were grown in plastic pots (15 cm in diameter and 13 cm in height), which were kept near the window in the rearing room at room temperature and watered every other day.

Host ranges of adults

Adults of *T. sordida* were collected from locality 5 (Fig. 1) on 7 June 2006, and adults of *T. nigrita* were collected from locality 15 (Fig. 1) on 13 and 18 June 2006. As a preliminary experiment, some adults were fed with leaves of *S. foetida* and *M. citrifolia*, and we confirmed that *T. nigrita* fed on both plants but *T. sordida* did not feed on *S. foetida*.

On 19 June 2006, four sets of five pairs of *T. sordida* males and females were used in the host range experiment, two sets of *T. sordida* were fed with one-third of a *M. citrifolia* leaf and the remaining two sets were fed with a *P. foetida* leaf as a control. Each set was put in a Petri dish and kept in an incubator at 22.5°C under L : D 14:10 conditions.

On 21 June 2006, three sets of five pairs of *T. nigrita* males and females were used in the host range experiment using *T. nigrita* with three *S. foetida* leaves, one-third of a *M. citrifolia* leaf, and a *P. foetida* leaf, respectively. Each set was put in a Petri dish and kept in an incubator at 25°C under L : D 14:10 conditions.

The number of *T. sordida* and *T. nigrita* adults surviving was recorded every day until the death of all adults fed with plant species other than *P. foetida* and thereafter every other day until the death of all adults fed with *P. foetida*.

Larval host ranges

Trachyaphthona sordida adults that had been collected from locality 6 (Fig. 1) on 10 June 2006 were kept in an incubator at 22.5°C under L : D 14:10 conditions. Eggs were kept in an incubator at 22.5°C under dark conditions. On 20 July 2006, 25 larvae hatched and were

transferred individually to a plastic box, which was kept in an incubator at 22.5°C. Ten larvae were fed with fine roots of *S. foetida* and the remaining 15 were fed with *M. citrifolia*. The fine roots were renewed every other day and larval development was observed at the same time.

In 2005, *T. nigrita* adults that had been collected from locality 18 (Fig. 1) on 29 June were kept under laboratory conditions. Eggs were kept in an incubator at 25°C. On 28 August 2005, ten larvae hatched and were transferred to Petri dishes containing soil and kept in an incubator at 25°C. Five larvae were fed with fine roots of *S. foetida* and the remaining five were fed with *M. citrifolia* roots.

In 2006, *T. nigrita* adults that had been collected from locality 15 on 18 June were kept in an incubator at 25°C under L : D 14:10 conditions. Eggs were kept in an incubator at 22.5°C. On 23, 28 and 30 July 2006, ten larvae hatched and were transferred individually to plastic boxes and kept in an incubator at 22.5°C. Five larvae were fed with fine roots of *S. foetida* and the remaining five were fed with fine roots of *M. citrifolia*.

RESULTS

Life history traits

Field surveys

Distribution in Kyushu. Trachyaphthona sordida was found in various localities in the mainland of Kyushu, except for the most southern part of the Satsuma and Ôsumi Peninsulas, Kagoshima Prefecture (Fig. 1). The highest elevation where *T. sordida* was found was Fukigoe (900 m a.s.l.), Obama Town, Nagasaki Prefecture. *Trachyaphthona nigrita* was found in the southern parts of the Satsuma and Ôsumi Peninsulas, Kagoshima Prefecture (Fig. 1). The highest elevation where *T. nigrita* was found was Mount Nomadake (591 m a.s.l.), Kasasa Town, Kagoshima Prefecture. We observed that *T. nigrita* and *T. sordida* coexist in the southern parts of the Satsuma Peninsula, Kagoshima Prefecture.

Seasonal occurrence of adults. In the northern parts of Kyushu, most *T. sordida* adults appeared from late May to late June (Fig. 2), but did not appear after August. In the southern parts of Kyushu, most *T. sordida* adults appeared from mid-May to mid-June (Fig. 2), but did not appear after August, except for a few individuals found in October.

Trachyaphthona nigrita adults started to appear in late April and reached a peak in June (Fig. 3). On 12 October 2006, one male was collected from Akime (locality 19 in Fig. 1) and it survived until 6 January 2007 under laboratory conditions. In addition, one female and one male were collected on 24 September 2006 and 20 October 2006, respectively, in outdoor rearing cages (1.8 × 1.8 × 1.8 m) that had been used for a preliminary cage experiment. The female survived until 7 November 2006 and the male until 29 October 2006.

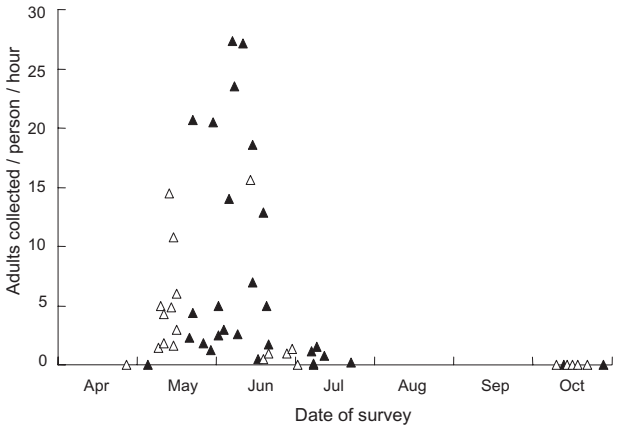

Figure 2 Seasonal changes in the number of *Trachyaphthona sordida* adults collected. Black and white symbols represent *T. sordida* collected from northern Kyushu and from southern Kyushu, respectively.

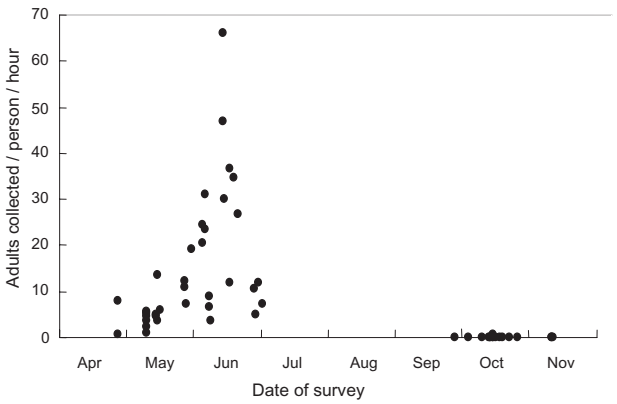

Figure 3 Seasonal changes in the number of *Trachyaphthona nigrita* adults collected.

Laboratory experiments

Development of Trachyaphthona *eggs and larvae.* Both *T. sordida* and *T. nigrita* females laid their eggs in the soil near the ground surface (2–5 mm in depth) and occasionally on the walls of the box. Larvae of

T. sordida and *T. nigrita* fed on the outer surface of fine *P. foetida* roots during all larval stadia.

Trachyaphthona sordida. There were three larval stadia. The number of days required for development of eggs, first instars and second instars under different temperature conditions decreased with increasing temperature (Table 1). The third larval stadium lasted for approximately a month and became a prepupa. The relationship between rearing temperature and developmental rate was explained well by a linear regression. Based on the best fit line for eggs ($y = 0.0067K_0 - 0.0659$, $R^2 = 0.9835$, $P < 0.01$), first instars ($y = 0.0036K_0 - 0.0183$, $R^2 = 0.9243$, $P < 0.01$) and second instars ($y = 0.0074K_0 - 0.0777$, $R^2 = 0.9465$, $P < 0.05$), we determined lower developmental thresholds and day degrees above the lower developmental thresholds for each stage (Table 2). For the first instar, the lower developmental threshold was lowest and the day degrees above the lower developmental threshold was highest.

Six (66.7%) of nine *T. sordida* third instars that had been kept at 25°C died before 23 October 2005 and the three remaining third instars (33.3%) pupated on 28 and 29 August 2005. All of them emerged from 10 to 13 September 2005. Nine (45%) of 20 *T. sordida* third instars that had been kept at 20°C died before 23 October 2005. Three of the 11 surviving individuals pupated during the period from 29 August to 21 October. Two of these emerged on 11 September and 16 October 2005 and one died. The eight remaining third instars became prepupae. Four (80%) of five *T. sordida* third instars that had been kept at 18°C died before 23 October 2005 and only one pupated on 19 September 2005 and emerged on 4 October 2005. Two of eight (25%) *T. sordida* prepupae pupated in late April and emerged in early May, and the others died before spring 2006. The number of third instars that pupated at higher temperatures was greater than at lower temperatures.

Trachyaphthona nigrita. Usually there were three larval stadia, but a few third instars molted into fourth instars at temperatures higher than 20°C and then into fifth instars at temperatures higher than 25°C. The numbers of days required for the development of eggs, first instars and second instars under different temperature conditions are shown in Table 1. Final larval stadia (usually third, rarely fourth or fifth larval stadia) lasted for approximately a month and proceeded to prepupae. The relationship between rearing temperature and developmental rate was explained well by a linear regression. Based on the best fit line for eggs

Table 1 Number of days required for development of each stage of *Trachyaphthona sordida* and *Trachyaphthona nigrita* at different temperatures

T. sordida				Temperature (°C)	*T. nigrita*		
Temperature (°C)	Egg	First instar	Second instar		Egg	First instar	Second instar
15	26.6 ± 0.6 (181)	26.0 ± 1.8 (5)		15	30.4 ± 0.9 (55)	37.0 ± 4.1 (2)	
18		24.9 ± 4.5 (7)	19.3 ± 1.3 (4)	18			23.0 ± 6.2 (2)
20	15.2 ± 0.2 (490)	17.5 ± 1.3 (17)	13.0 ± 0.8 (10)	20	17.4 ± 0.2 (298)	24.2 ± 2.4 (15)	18.4 ± 2.2 (7)
22	12.7 ± 0.5 (71)	16.0 ± 2.3 (11)	12.5 ± 1.0 (6)	22.5	14.4 ± 0.3 (133)	23.3 ± 1.1 (27)	17.1 ± 1.0 (14)
25	9.4 ± 0.2 (445)	14.0 ± 1.3 (13)	9.3 ± 2.0 (7)	25	9.0 ± 0.2 (168)	16.6 ± 1.4 (21)	11.8 ± 1.3 (10)

Data are mean no. days ± SD. Numbers in parentheses indicate the number of individuals examined.

Table 2 Developmental threshold and day degrees above the developmental threshold for each stage of *Trachyaphthona sordida* and *Trachyaphthona nigrita* development

	T. sordida			*T. nigrita*		
	N	Developmental threshold (°C)	Day degrees above developmental threshold	N	Developmental threshold (°C)	Day degrees above developmental threshold
Egg	1187	9.8	149.3	654	11.3	138.9
First instar	53	5.1	277.8	65	6.6	333.3
Second instar	27	10.5	135.1	33	10.3	181.8

($y = 0.0072K_0 - 0.0815$, $R^2 = 0.8919$, $P = 0.056$), first instars ($y = 0.003K_0 - 0.0198$, $R^2 = 0.9097$, $P < 0.05$) and second instars ($y = 0.0055K_0 - 0.0566$, $R^2 = 0.8996$, $P < 0.05$), we determined lower developmental thresholds and day degrees above the lower developmental thresholds for each stage (Table 2). For the first instar, the lower developmental threshold was lowest and the day degrees above the lower developmental threshold was highest.

One (10%) of ten *T. nigrita* third instars that had been kept at 25°C died before 23 October 2005. One (11.1%) of the nine remaining third instars pupated on 28 August 2005 and emerged as an adult on 9 September 2005. The eight (88.9%) remaining third instars molted into fourth instars, four of which became prepupae and the others molted into fifth instars. One of the four fifth instars died and the others became prepupae. Three (30%) of ten *T. nigrita* third instars that had been kept at 20°C died before 23 October 2005. Two (28.6%) of the seven third instars developed into fourth instars and became prepupae. The five (71.4%) remaining third instars became prepupae. One of three *T. nigrita* third instars that had been kept at 18°C died and the two remaining third instars became prepupae.

All prepupae derived from the three fifth instars and the two fourth instars died before spring 2006. Five of seven (71%) prepupae derived from the third instars also died before spring 2006, and the two (29%) remaining prepupae survived until the end of April or early May 2006, but they died before pupation.

Amounts of P. foetida *roots eaten by* Trachyaphthona *larvae.* In total, *T. sordida* fed on 1117.4 ± 129.1 mm^3 of *P. foetida* roots during the period from first to third larval stadia. *Trachyaphthona nigrita* fed on 719.4 ± 149.1 mm^3 of *P. foetida* roots during the period from first to third larval stadia, and 1714.4 ± 240.5 mm^3 during the entire larval stage, including the fourth larval stadium (Table 3).

Table 3 Amounts of *Paederia foetida* roots eaten by *Trachyaphthona sordida* and *Trachyaphthona nigrita* larvae

	T. sordida		*T. nigrita*	
	n	Amount (mm^3)	*n*	Amount (mm^3)
First instar	16	79.4 ± 9.1	27	64.0 ± 15.5
Second instar	5	263.8 ± 81.7	14	189.6 ± 39.0
Third instar	2	774.2 ± 38.3	11	471.8 ± 94.6
Fourth instar	–	–	4	995.0 ± 91.4

Data are mean ± SD.

Host specificity

Host ranges of adults

Trachyaphthona sordida adults did not feed on *S. foetida* leaves and died within a day in a preliminary experiment, hence a rearing experiment was not performed with *S. foetida*. *Trachyaphthona sordida* adults fed with *M. citrifolia* died an average of 1.6 days earlier than those fed with *P. foetida* (Fig. 4), but there was no significant difference in the mean longevity of adults fed with *P. foetida* (mean ± SD: 6.45 ± 1.65 days) and *M. citrifolia* (4.85 ± 1.18 days; d.f. = 19, $t = 1.67$, $P = 0.2$).

Trachyaphthona nigrita adults fed on leaves of both *M. citrifolia* and *S. foetida*. Adults that were fed with *M. citrifolia* died after 3.6 days on average. Those fed with *S. foetida* died soon after the beginning of the experiment, but a few adults survived for as long as those fed with *P. foetida* (Fig. 4). The mean longevity of adults fed with *M. citrifolia* (mean ± SD: 3.60 ± 1.65 days) and *S. foetida* (9.10 ± 9.39 days) was significantly shorter than that of adults fed with *P. foetida* (16.60 ± 10.57 days; d.f. = 9, $t = 4.49$, $P = 0.005$ and d.f. = 9, $t = 3.77$, $P = 0.005$, respectively).

Larval host ranges

All *T. sordida* first instars fed on the fine roots of both *M. citrifolia* and *S. foetida*, but those fed with the fine

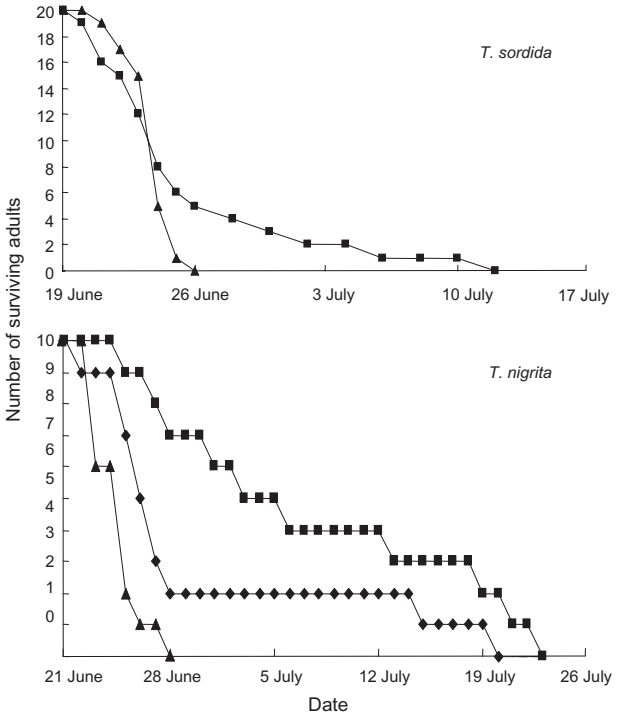

Figure 4 Changes in the number of surviving *Trachyaphthona sordida* and *Trachyaphthona nigrita* adults fed with *Paederia foetida*, *Serissa foetida* and *Morinda citrifolia*. Plant species: (■), *P. foetida*; (▲), *M. citrifolia*; (◆), *S. foetida*.

roots of *M. citrifolia* died before molting into second instars. Only 30% of first instars ($n = 10$) that fed on the fine roots of *S. foetida* progressed to second instars. All second instars molted into third instars, but only two of the third instars became prepupae, and the prepupae did not progress to pupae.

In experiments in both 2005 and 2006, all first instars fed with the fine roots of *M. citrifolia* died before molting into second instars. Only 40% ($n = 5$) and 60% ($n = 5$) of the first instars fed with *S. foetida* progressed into second instars in 2005 and 2006, respectively. In the 2005 experiment, one of the two second instars died at the second larval stadium, but the one remaining individual molted into a third instar in September and further into a fourth instar in October. This larva overwintered normally, pupated and emerged in May 2006. In the 2006 experiment, all of the three second instars molted into third instars in September and further into fourth instars in October and November. Two of these died before becoming prepupae and the one remaining fourth instar became a prepupa in November, but the prepupa died in December before pupation.

DISCUSSION

Patterns of adult appearance for *T. nigrita* in southern Kyushu and *T. sordida* in both southern and northern Kyushu (Figs 2,3) indicate that both species are fundamentally univoltine. Adults of both species are present from late spring to early summer and lay eggs near *P. foetida* roots just below the soil surface. The egg stage and first and second larval stadia last for approximately two weeks each. Third instars or prepupae may overwinter in the soil, pupate the following spring, and emerge as adults in late spring (Fig. 5).

One *T. nigrita* male was collected from Bounotsu Town on 12 October 2006 and survived until 6 January 2007. This male is regarded as an autumn emergent adult, because a few adults emerged in August and September under rearing conditions in the laboratory, and two adults were found in September and October in outdoor rearing cages. These observations suggest that a few individuals of *T. nigrita* may overwinter as adults.

Trachyaphthona nigrita larvae reared in the laboratory exhibited developmental polyphenism, which is common in insects (Crowson 1981). Polyphenism of larval development in which extra larval instars occur is influenced by nutrition (Schmidt & Lauer 1977), photoperiod (Fantinou *et al.* 1996), temperature, relative humidity (Schmidt & Lauer 1977) and genetic factors (Gold *et al.* 1999). In the case of *T. nigrita*, the number of larval stadia varied with rearing temperature, which seemed to be one of the most important factors affecting polyphenism. In addition, intrinsic factors are also considered to influence the larval polyphenism of *T. nigrita*, because larvae reared at the same temperature exhibited developmental polyphenism.

Trachyaphthona sordida adults have never been found after August in the field, but some *T. sordida* pupated and emerged before winter under laboratory conditions. This suggests that exposure to a low winter temperature is not necessary for *T. sordida* to emerge. Larvae of *T. sordida* did not exhibit developmental polyphenism.

The lower developmental threshold of *T. sordida* was lower than that of *T. nigrita* (Table 2). This might be related to the fact that *T. sordida* is distributed in more northern parts of Kyushu than *T. nigrita*, and adults appear earlier than *T. nigrita* in southern Kyushu where the two species coexist.

Preliminary host range experiments indicate that *M. citrifolia* is not an acceptable host plant to either *T. sordida* or *T. nigrita*, whereas *S. foetida* is acceptable to adults and larvae of *T. nigrita* and larvae of *T. sor-*

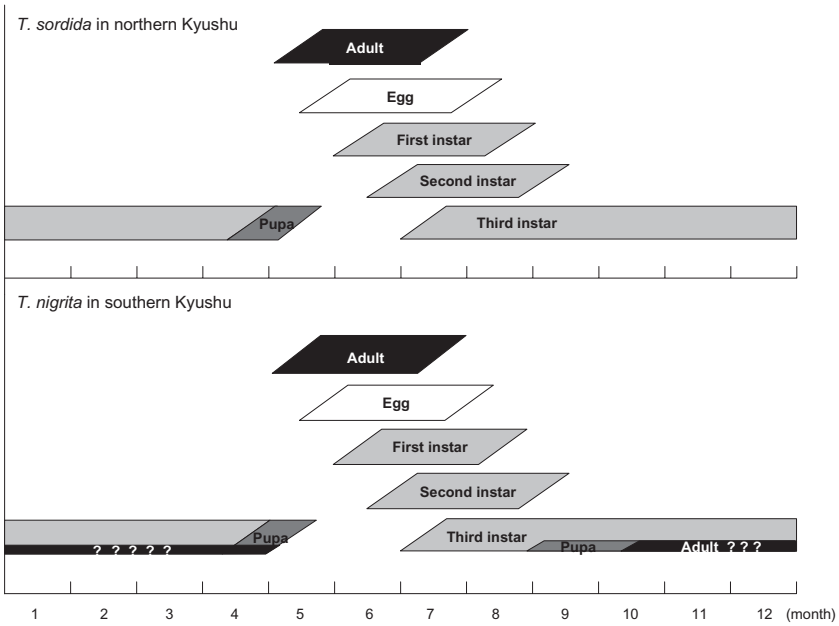

Figure 5 Schematic representation of the life history of *Trachyaphthona sordida* in northern Kyushu and *Trachyaphthona nigrita* in southern Kyushu. Question marks indicate unknown parts of this species' life history.

dida. Both species of *Trachyaphthona* beetles may have tribe Paederiae-level host specificity, but many other plants need to be tested to define their host ranges because many other tribes in the Rubiaceae are represented in Hawaii and Florida.

The total amount of root eaten by larvae as estimated in the present study might be a slight underestimate, because some *P. foetida* roots were occasionally completely eaten by a larva before being renewed, which suggests that that larva could have eaten more. On the basis of their life history traits and host specificity, we conclude that both *T. sordida* and *T. nigrita* appear to have potential as biological control agents against skunk vine.

In Makurazaki (13 in Fig. 1; 31°16′N, 130°17′E), where *T. nigrita* is distributed, the absolute coldest temperature in January has ranged from –3.1 to 1.3°C over the past 45 years (Japan Meteorological Agency 2006). In central Florida, the absolute coldest temperature in January has ranged from –6.7 to 5.6°C over the last 46 years at Saint Leo (28°34′N, 82°26′W), near Tampa, where *P. foetida* is most severe (W. Schmitz, Service Climatologist/Meteorologist, South-east Regional Climate Center, pers. comm., 2006). Comparison of the absolute coldest temperatures of Makurazaki and Saint Leo indicates that central Florida is milder than southern Kyushu, which suggests that *T. nigrita* should be able to tolerate the winter temperature. Based on this climatic tolerance, its root feeding and the preliminary host specificity testing, *T. nigrita* appears to have potential as a biological control agent in Florida and possibly in subtropical areas such as Hawaii.

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REFERENCES

Bremer B, Manen JF (2000) Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). *Plant Systematics and Evolution* **225,** 43–72.

Crowson RA (1981) *The Biology of the Coleoptera*. Academic Press, London.

Fantinou AA, Tsitsipis JA, Karandinos MG (1996) Effect of short- and long-day photoperiods on growth development of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *Environmental Entomology* **25,** 1337–1343.

Frick KE (1970) *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae), a flea beetle for the biological control of tansy ragwort. 1. Host plant specificity studies. *Annals of the Entomological Society of America* **63,** 284–296.

Gann G, Gordon D (1998) *Paederia foetida* (skunk vine) and *P. cruddasiana* (sewer vine): threats and management strategies. *Natural Areas Journal* **18,** 169–174.

Gassmann A, Schroeder D, Maw E, Sommer G (1996) Biology, ecology, and host specificity of European *Aphthona* spp. (Coleoptera, Chrysomelidae) used as biocontrol agents for leafy spurge *Euphorbia esula* (Euphorbiaceae), in North America. *Biological Control* **6,** 105–113.

Gold CS, Nemeye PS, Coe R (1999) Recognition and duration of the larval instars of banana weevil, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae), in Uganda. *African Entomology* **7,** 49–62.

Japan Meteorological Agency (2006) *Information on Meteorological Statistics: Meteorological Observation*. Available from URL: http://www.data.kishou.go.jp/etrn/index.html (In Japanese.)

Julien MH, Griffiths MW (1998) *Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds*, 4th edn. CAB International, Wallingford, UK.

Kimoto S (1966) The Chrysomelidae of Japan and the Ryukyu islands. X Subfamily Alticinae III. *Journal of the Faculty of Agriculture, Kyushu University* **13,** 601–633.

Kimoto S, Takizawa H (1994) *Leaf Beetles (Chrysomelidae) of Japan*. Tokai University Press, Tokyo. (In Japanese.)

Kobayashi H (2000) *Life of Leaf Beetles (Chrysomelidae): Guideline for Insect Study*. Shinmai Shoseki Shuppan Center, Nagano. (In Japanese.)

Langeland KA, Burks KC, eds. (1998) *Identification and Biology of Non-Native Plants in Florida's Native Areas*. University of Florida, Gainesville, FL.

Luken JO, Beiting SW, Kareth SK, Kumler RL, Liu JH, Seither CA (1994) Target and non-target discrimination of herbicides applied to vegetation in a power-line corridor. *Environmental Management* **18,** 251–255.

Morton JF (1976) Pestiferous spread of many ornamental and fruit species in south Florida. *Proceedings of the Florida Horticultural Society* **89,** 348–353.

Ohno M (1961) On the species of the genus *Trachyaphthona* Heikertinger and the new genus *Sphaeraltica* (Coleoptera, Chrysomelidae). Studies on the flea-beetles of Japan (3). *Bulletin of the Department of Liberal Arts, Toyo University* **2,** 73–92.

Ohno M (1966) Notes on *Aphthona*-species occurring in Japan. *Suzumushi* **16,** 26–31. (In Japanese.)

Pemberton RW, Pratt PD (2002) Skunk vine (*Paederia foetida*). In: Van Driesche R, Blossey B, Hoddle M, Lyon S, Reardon R (eds). *Biological Control of Invasive Plants in the Eastern United States*, pp 343–351. US Forest Service, Morgantown, WV.

Puff C (1991) Revision of the genus *Paederia* in Asia. In: Puff C (ed.). *Opera Botanica Belgica 3: The genus Paederia L. (Rubiaceae-Paederieae): A multidisciplinary study*, pp 207–289. National Botanic Garden of Belgium, Meise.

Schmidt FH, Lauer WL (1977) Developmental polymorphism in *Choristoneura* spp. (Lepidoptera: Tortricidae). *Annals of the Entomological Society of America* **70,** 112–118.

Stocker R, Brazis D (1999) *Current Control Technology for the Control of Skunk Vine (Paederia foetida)*. Final Report 34. Center of Aquatic Plants, Institute of Aquatic Plants, University of Florida, Gainesville, FL.

Yamazaki T (1993) Rubiaceae. In: Iwatsuki K, Yamazaki T, Boufford DE, Ohba H (eds). *Flora of Japan IIIa*, pp 206–240. Kodansha, Tokyo.